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Modulation of acute transient exercise-induced hypertension after oral administration of four angiotensin-converting enzyme inhibitors in normotensive horses

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ABSTRACT

Changes in blood pressure (BP) during acute hypertension in response to angiotensin-converting enzyme inhibitors (ACEIs) have not been investigated in normotensive horses. In this study, six healthy horses were subjected to five trials, consisting in a treadmill exercise workload of 8 m/s for 1 min, 2 h after oral administration (PO) of placebo (0 mg/kg), enalapril (2.0 mg/kg), quinapril (1.0 mg/kg), ramipril (0.2 mg/kg) or benazepril (0.5 mg/kg). Serum angiotensin converting enzyme (ACE) activity was measured and systolic (SBP) and diastolic (DBP) blood pressures were recorded at rest (R), 2 h after placebo or ACEI administration (pre-E) and within the first 20 s after exercise (post-E).

Mean maximum serum ACE inhibition 2 h after PO administration was 4.8% (placebo), 39.4% (enalapril), 46.4% (quinapril), 55.0% (ramipril) and 71.68% (benazepril). There were no significant differences in serum ACE inhibition between enalapril and quinapril. SBP and DBP at times R and pre-E were not different in any of the five trials. In response to exercise, SBP increased by 67.6% (placebo), 52.7% (enalapril), 43.1% (quinapril), 26.6% (ramipril) and 4.2% (benazepril). In response to exercise, DBP increased by 20.6, 13.2, 11.7, 16.6 and 3.7% after placebo, enalapril, quinapril, ramipril and benazepril administration, respectively. Serum ACE activity changed during exercise, but statistical significance was not achieved. In conclusion, administration of PO benazepril at a dose of 0.5 mg/kg modulated physiological hypertension induced by exercise in horses that were otherwise normotensive.

Introduction

Angiotensin-converting enzyme inhibitors (ACEIs) prevent conversion of angiotensin I to II and attenuate the actions of angiotensin II (Brewster et al., 2003; Toutain and Lefebvre, 2004; Atlas, 2007). Angiotensin II activates AT1 receptors on vascular smooth muscle of pre-capillary arterioles and post-capillary venules, causing vasoconstriction (Brewster et al., 2003; Atlas, 2007). ACEIs cause mixed vasodilatation, by decreasing available angiotensin II and sympathetic release of adrenaline, and by enhancing available bradykinin and bradykinin-mediated release of nitric oxide (Muir et al., 2001). ACEIs are administered per os (PO) as pro-drugs (i.e., benazepril), which are more lipophilic and better absorbed from the gastrointestinal tract than the active drugs (i.e. benazaprilat). Pro-drugs are subsequently transformed to their active metabolites by esterases and inhibit angiotensin converting enzyme (ACE) (Toutain and Lefebvre, 2004).

The effects of ACEIs on blood pressure (BP) in normotensive human beings or animals have not been completely elucidated, with discrepant results between studies. In normotensive human beings, Gainer et al. (1998) did not find significant changes in systolic (SBP), diastolic (DBP) and mean (MBP) blood pressures after ACEI administration (captopril). In other studies, BP decreased in normotensive humans when they were sodium-depleted (Vidt et al., 1982; Todd and Heel, 1986). Several studies have shown that acute changes in BP are correlated with pre-treatment plasma renin activity and angiotensin concentrations, with the greatest reductions in BP in patients with the highest plasma renin activity (Waeber et al., 1982; Given et al., 1984). Conversely, Shoback et al. (1983) reported that administration of an ACEI (enalapril) lowered BP in normotensive humans in a dose-related fashion.

These differences between studies in the effects of ACEIs on BP have been also observed in normotensive horses; PO administration of 0.5–2.0 mg/kg enalapril did not influence BP (Gardner et al., 2004; Gómez-Díez et al., 2014). Davis et al. (2014) did not find

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significant changes in BP after PO administration of 0.25–0.5 mg/kg quinapril. The lack of vasodilatory effects of ACEIs was attributed to normotension and to a non-activated renin–angiotensin–aldosterone axis. In contrast, Afonso et al. (2013) observed a mild reduction in BP (5–10 mm Hg) after PO administration of low and high doses of ramipril, quinapril, benazepril and peridonpril.

The effects of ACEIs on BP in normotensive horses during an acute hypertensive physiological situation have not been investigated. We used an experimental model of temporal hypertension based on exercise; brief, strenuous exercise in horses induces a dramatic and sudden rise in BP (Masri et al., 1990). In the current study, four different ACEIs and placebo were administered PO before exercise; BP and inhibition of serum ACE activity were measured before and after exercise. The main aims of the study were: (1) to elucidate whether pre-exercise PO administration of an ACEI reduces the hypertensive response to exercise, and (2) to assess whether the changes in BP after exercise are associated with inhibition of circulating/serum ACE activity. It was hypothesised that the acute transient hypertensive response to exercise would be lower after PO ACEI administration and that this effect would be proportional to the degree of inhibition of serum ACE activity.

Materials and methods

Horses

Six healthy mature unfit crossbred horses (three mares and three geldings), aged 8–12 years and with body weights of 382–525 kg, were included in the study. The horses were found to be healthy on physical examination, haematology and serum biochemical profile, BP monitoring and echocardiography. During the experiments, the horses were housed outdoors, in paddocks, with free access to water, but not to salt blocks. They were fasted for 12 h prior to and 8 h after PO administration of ACEIs/placebo, even though it has been demonstrated that feeding does not affect serum ACE inhibition (Afonso et al., 2013). On days outside the experiment, horses were maintained on their normal ration of ryegrass haylage.

Experimental design

A blinded and randomised Latin square design in five trials was used. Two hours before exercise, each animal received a single PO dose of placebo or ACEI (2.0 mg/kg enalapril, 1.0 mg/kg quinapril, 0.2 mg/kg ramipril or 0.5 mg/kg benazepril). These doses and the time between dosage and exercise were selected according to the results of previous ACEI pharmacokinetics–pharmacodynamics (PK-PD) studies, which found that maximum serum ACE inhibition occurs at ~2 h (Afonso et al., 2013; Davis et al., 2014; Gómez-Díez et al., 2014, unpublished observations from our group). There was a washout period of 1 week between each experiment. The study was approved by the Ethical Committee for Animal Experimentation of the University of Córdoba (approval number 55.60 PE; date of approval 1 February 2010).

Preparation of angiotensin-converting enzyme inhibitors

Enalapril hydrochloride (Enacard 20 mg, Merial), ramipril (Vasotop P 5 mg, Merck Sharp and Dohme Animal Health), quinapril hydrochloride (Acuprel 40 mg, Pfizer) and benazepril hydrochloride (Fortekor 20 mg, Novartis) were used in the study. For each pro-drug, tablets were dissolved and suspended in 150 mL of water, sonicated in an ultrasonic bath for 15 min and stored at 4 °C before trials. For placebo dosing, the equivalent amount of water without pro-drug was used. Pro-drug and placebo administration was made by nasogastric intubation, then the nasogastric tube was rinsed with 250 mL of water.

Treadmill exercise

Horses were subjected to a brief intense treadmill exercise (Mustang 2000, Kagra) to induce hypertension. The exercise was preceded by 5 min at walk (1.3–1.5 m/s) and 5 min at trot (3.5–3.7 m/s), on the treadmill without inclination. Intense exercise consisted of a workload of 8 m/s for 1 min, with the treadmill inclined by 6%. The horses were unfit and untrained and, therefore, this exercise protocol was of sufficient intensity to induce hypertension. After exercise, horses performed a cooling-down exercise, with 5 min at trot and 5 min at walk on the treadmill uninclined.

Collection of blood samples

Venous blood samples were collected and BP was measured three times: at rest, before starting the experiments, with horses in the paddock (R), 2 h after PO administration of placebo or ACEIs (pre-exercise, pre-E) and within the first 20 s after intense exercise (post exercise, post-E). Blood samples allowed to coagulate in plain tubes in a refrigerator for 30 min, then centrifuged and the serum was stored at –90 °C until analysis.

Measurement of angiotensin-converting enzyme inhibitor activity

Serum ACE activity was quantified by the hydrolysis of N-[3-(2-furyl) acrylolyl]-L-phenylalanyl-glycyl-glycine (FAPGG), forming furylacryloyl-phenylalanine (FAP), which results in a decrease in absorbance at 340 nm (Maguire and Price, 1985). This method has been used to measure serum ACE activity in horses previously (De Mello Costa et al., 2010, 2012; Costa et al., 2011; Afonso et al., 2013; Gómez-Díez et al., 2014). FAPGG (500 µL) was mixed with 50 µL serum and incubated for 5 min at 37 °C. The absorbance was measured at 340 nm with a spectrophotometer (Biosystems, model A-15). Quality control was performed using specific reagents for this assay. Linear calibration curves from 3 to 150 IU/L were obtained; the correlation coefficients (r) were >0.99, the precision was 2.86–6.21% and the limit of quantification (LOQ) and limit of detection (LOD) were 5 and 3 IU/L, respectively.

Measurement of blood pressure

Systolic and diastolic blood pressures (SBP and DBP, respectively) were measured non-invasively from the coccygeal artery (S/5 Datex-Ohmeda CompactR). Three measurements were made during R and pre-E; results are presented as the mean of each of the three recordings. At post-E, only one measurement was made, because of the rapid decrease in BP after exercise.

Statistical analysis

Descriptive statistical parameters including arithmetic mean, maximum and minimum were calculated. The Kruskal–Wallis test was used to compare differences for each parameter measured at the three time points (R, pre-E, post-E). When significant differences were found, a Wilcoxon rank sum test was used as a second test (pairwise comparison). Correlations between the different parameters were calculated using Spearman's rank correlation. The significance level was set at $P < 0.05$. The statistical software was Statgraphics Centurion XVI.I for Windows (StatPoint Technologies).

Results

The six horses tolerated PO administration of the four ACEIs without any noticeable adverse effects. Serum ACE activity and SBP and DBP values for the five trials and for the three sampling times are presented in Figs. 1–3, respectively. The baseline ACE activity before administration of ACEI or placebo did not change over the time of study between trials ($P = 0.53$) or between animals ($P = 0.58$). Differences in SBP and DBP at time R were not found between trials and the horses were normotensive. Two hours after PO administration (time pre-E), serum ACE activity did not change in the placebo trial, but decreased in the four ACEI trials, achieving the greatest reduction after PO benazepril (Fig. 1). At this time, all the horses were normotensive and a significant effect on BP was not detected.

There was no significant increase in serum ACE activity with exercise in any of the five trials. Exercise after placebo administration induced a marked increase in SBP and a mild increase in DBP (Figs. 1–3). The exercise-induced rise in SBP was reduced when ACEIs were administered PO, and the maximal reduction was achieved in the benazepril trial.

The percentages of serum ACE inhibition and increases in SBP and DBP, comparing post-E and R values for each trial, are presented in Table 1. The percentage of serum ACE inhibition at time post-E compared to R time was negatively correlated with the percentage of increase in SBP ($r = –0.861$), but correlations with the percentage of increase presented by DBP were not significant ($r = –0.270$).

Discussion

The aims of this study were to elucidate whether PO administration of different ACEI pro-drugs, prior to the exercise, could modulate the physiological hypertensive response to exercise and whether the reduced response of the BP to intense exercise was associated with the degree of inhibition of serum ACE activity. The